REMARKS

Claims 1-6, 8, 9, 11-16, 18, 19, 21, 31, 32 and 36-65 are pending in this application following entry of the amendment above. Claims 35 and 59 are canceled, Claims 1, 8, 9, 12, 18, 19, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54 and 56 are amended. Claims 62-65 have been added. A marked up version of the claim amendments is attached hereto and is captioned "Version with Markings to Show Changes Made." As requested by the Examiner, a clean set of the entire pending claim set is also enclosed herewith. However, for the purposes of entering the claim amendments into the record, the amendments set forth above, and not the clean copy of the claim set, should be utilized.

The claims stand rejected for lack of written description, non-enablement and/or anticipation. Claim 35 had been objected to for informalities. This rejection has been obviated by canceling Claim 35 as drawn to a non-elected invention. The individual rejections will be addressed below in the order presented in the outstanding Office Action.

I. Support for Claim Amendments.

The amendments presented above have been made to correct minor errors in the claims or to point out particular features of the inventions so as to expedite the prosecution of the present application to allowance in accordance with the USPTO Patent Business Goals (65 Fed. Reg. 54603, September 8, 2000). These amendments to not represent an acquiescence or agreement with any of the outstanding rejections.

Claim 1 has been amended to recite that the geminivirus silencing vector comprises the "geminivirus AL1, AL2 and AL3 coding sequences." This recitation is supported by the specification, *e.g.*, the working examples, which describe geminivirus silencing vectors encoding AL1, AL2 and AL3.

Claim 1 has also been amended to recite "wherein said heterologous DNA is constitutively expressed and said AL1, AL2 and AL3 coding sequences are bidirectionally transcribed from said geminivirus silencing vector." This recitation is supported by the specification in Figure 1A and at

page 8, lines 20-23, which recites "[o]n both components, the ORFs diverge from a conserved 230 nucleotide intergenic region (common region) and are transcribed bidirectionally from double stranded replicative form DNA. The ORFs are named according to genome component and orientation relative to the common region (i.e., left versus right)." It is apparent that the viral bidirectional transcription apparatus is being maintained in the silencing vectors described in the working examples, *e.g.*, the heterologous sequence is being transcribed in the rightward direction by the AR1/coat protein promoter and AL1, AL2 and AL3 are being transcribed in the leftward direction by the AL1 promoter, which lies partially in the common/intergenic region. This recitation is also supported by the specification at page 6, lines 6-9, and the working examples, which describe constitutive expression of luciferase and *su* silencing sequences.

Claim 1, as well as Claims 8, 9, 12, 18, 19, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54 and 56 have been amended to recite "at least 80% sequence similarity." Accordingly, Claim 59 has been canceled. This recitation is supported by the application at page 11, lines 3-6.

Claims 38 and 40 have been amended to recite "said heterologous DNA having at least 60% sequence similarity to a coding region of a gene endogenous to a plant, wherein the heterologous DNA sequence is inserted into the silencing vector in the antisense orientation." This recitation is supported by the working examples and the specification, *e.g.*, at page 12, lines 26-29.

Claims 40, 54 and 56 have been amended to correct minor errors.

New Claim 62 is similar to Claim 1, except that Claim 62 specifically recites "TGMV". In addition, Claim 62 recites that the AL1, AL2 and AL3 coding regions are "operably associated with an AL1 promoter." This recitation is supported by the working examples and Figure 1A; those of ordinary skill in the art would appreciate that the constructs used to carry out the working examples drove expression of AL1, AL2 and AL3 by the geminivirus AL1 promoter. Finally, Claim 62 further recites that the heterologous DNA is

"operably associated with a geminivirus coat protein promoter." This recitation is supported throughout the specification, including originally filed Claim 5.

New Claim 63 recites a method of gene silencing and is similar to Claim 50, except that new Claim 63 depends from Claim 42, which specifically recites TGMV.

New Claim 64 recites a method of gene silencing comprising "providing a nucleic acid sequence encoding the geminivirus movement proteins to said plant cell." This recitation is supported throughout the specification, including the working examples, which describe methods of gene silencing that involving providing the geminivirus BL1 and BR1 movement proteins. Dependent Claim 65 specifically recites that the geminivirus silencing vector is derived from TGMV and the plant cell "is a cell from a species of *Nicotiana*." TGMV silencing vectors are supported throughout the specification. Gene silencing in a *Nicotiana* cell is supported by the specification at page 17, line 5, which states that the invention may be carried out in "tobacco," and more specifically states in "*Nicotiana tabacum,*" and in the working examples which describe silencing in *Nicotiana benthamiana*.

In view of the foregoing, it is submitted that the claim amendments are supported by the application as filed, and the Applicant respectfully requests entry thereof.

II. Written Description.

The claims stand rejected under 35 U.S.C. § 112, paragraph 1 for lack of written description in the application as filed. The Applicant respectfully disagrees.

As described in more detail in Applicant's previous response, "[t]here is a strong presumption that an adequate written description of the claimed invention is present in the specification as filed." (Guidelines for Examination of Patent Applications Under the 35 U.S.C. 112, first paragraph, "Written Description" Requirement, 66 Fed. Reg. 1099, 1105 (Jan. 5, 2001)). The

present application is not one of the "rare" instances in which rejection of originally claimed subject matter is rejected for lack of written support. It is the Patent Office that "has the initial burden, after a thorough reading and evaluation of the content of the application, of presenting evidence or reasons why a person skilled in the art would not recognize that the written description of the invention provides support for the claims." (*Id.*) This burden has not been satisfied by the present rejection.

Moreover, a reading of the instant rejection as set forth in the Office Action suggests that the Examiner's concerns relate more to enablement (addressed below). The Examiner appears to be questioning the sufficiency of guidance provided by the specification so that one skilled in the art can make and use the invention without undue burden, which is the touchstone for enablement, not written description.

The Applicant respectfully contends that the specification does provide a sufficient written description so that one skilled in the art would appreciate that the Applicant was in possession of the claimed invention at the time of filing. Adequate written support does not require that the application contain an exhaustive enumeration of all possible geminiviruses and transgenes. Such information is readily available in the art, and, in any case, it would not be feasible to provide a listing of all transgenes that can be used according to the present invention. Likewise, the genomic organization of various geminiviruses is known (*see, e.g.*, BERNARD N. FIELDS *et al.*, VIROLOGY, volume 1, chapter 19 (4th ed., Lippincott-Raven Publishers), at pages 576-582). The USPTO itself has cautioned that "[t]he absence of definitions or details for well-established terms or procedures should not be the basis of a rejection under 35 U.S.C. § 112, para. 1, for lack of adequate written description" (Revised Interim Written Description Training Examples, "Synopsis of Application of Written Description Guidelines").

The independent claims have been amended to specifically recite "at least 80% sequence similarity." It is not necessary or feasible to list all

possible sequences having at least 80% sequence similarity (or 90% or 95%) to the gene to be silenced. Methods of determining sequence similarity and of obtaining degenerate sequences are well-known and readily available in the art. One skilled in the art would be able to readily envision a genus of nucleotide sequences having the recited level of sequence similarity to a target endogenous plant gene.

With respect to target genes, the Specification presents working examples with a number of constructs derived from the *su* (magnesium chelatase) and *luc* (luciferase) genes. The Applicant has since obtained similar data with a number of other genes. The Office Action fails to explain why it is believed that gene silencing by a geminivirus vector is unique to these two particular genes and constructs. If the present rejection is maintained, it is requested that the Examiner indicate why these two genes (one a transgene and one an endogenous plant gene) are so unique that the results obtained therewith cannot be considered representative of other plant genes. The Applicant contends that one skilled in the art would find the Applicant to be in possession of a method of silencing any plant gene in view of the results presented with the *su* and *luc* genes presented in the working examples.

Moreover, as described above, the independent claims have been amended to recite at least 80% sequence similarity (previously recited by Claim 59). With respect to Claims 60 and 61, these claims specifically recite that the silencing sequence has at least 90% or 95% sequence similarity. Accordingly, the claims recite a genus of nucleotide sequences having close structural similarity, which may be readily envisioned by the skilled worker. The Office Action does not specifically address these recitations, *e.g.*, in canceled Claim 59 (reciting at least 80% sequence similarity) and in Claims 60 and 61 (reciting at least 90% and 95%, respectively, sequence similarity). If the present rejection for lack of written description is maintained, it is respectfully requested that the Examiner address these arguments so that

Applicant may respond more completely to the Examiner's concerns and to provide a more complete record in the event of an appeal.

Further, the USPTO has specifically addressed the issue of written support for a genus of DNA molecules having a high degree of structural similarity. To illustrate, Example 9 ("Hybridization") of the Revised Interim Written Description Training Examples addresses a situation similar to that raised in the instant Office Action. In Example 9, the exemplary claim recites an "isolated nucleic acid that specifically hybridizes under highly stringent conditions to the complement of the sequence set forth in SEQ ID NO:1." The application used in Example 9 discloses a single cDNA (SEQ ID NO:1) which encodes a protein that binds to the dopamine receptor and stimulates adenylate cyclase activity. In the "Analysis" section for this Example, it is stated:

> Now turning to the genus analysis, a person of skill in the art would not expect substantial variation among species encompassed within the scope of the claims because the highly stringent hybridization conditions set forth in the claim yield structurally similar DNAs. Thus, a representative number of species is disclosed, since highly stringent hybridization conditions in combination with the coding function of DNA and the level of skill and knowledge in the art are adequate to determine that applicant was in possession of the claimed invention.

Likewise, the present claims recite molecules having a high degree of structural similarity. The independent claims have been amended to recite that the silencing sequence has at least 80% sequence similarity to the target endogenous plant gene. Claims 60 and 61 recite that the silencing sequence has at least 90% or 95% sequence similarity, respectively, to the target endogenous plant gene. Accordingly, Applicant submits that the description in the specification in view of the high degree of structural similarity among the recited nucleic acid molecules and the level and skill and knowledge in the art provides an adequate written description for silencing sequences having the recited levels of sequence similarity.

Thus, the pending claims are directed to a genus of molecules having close structural and functional similarity, such that one skilled in the art would recognize that Applicant was in possession of the claimed invention at the time of filing.

Finally, one skilled in the art would recognize and appreciate that complete sequence similarity will not be required to accomplish the present invention. Indeed, there was not complete sequence similarity between the *su* silencing vector (*su* sequences from *N. tabacum*) and the endogenous magnesium chelatase gene (in *N. benthamiana*) used in the working examples. Further, it is appreciated in the art that hybridization of shorter nucleotide sequences requires a higher degree of sequence homology; a lower degree of homology is generally tolerated by longer sequences. One skilled in the art would appreciate that the present invention may be practiced with the recited levels of sequence similarity; however, for optimal methods, the degree of sequence similarity will be greater with shorter silencing sequences.

The Applicant further asserts that the present specification provides adequate guidance for the claimed invention. The present specification states that any geminivirus may be used. TGMV is used as an illustrative virus. The silencing sequence may be expressed from anywhere in the geminivirus genome that allows expression (*e.g.*, transcription) and is not lethal to the virus (recognizing that some viral functions may be provided in trans from the cell or another construct). One exemplary embodiment that is describes involves substituting part or all of the viral sequences encoding the coat protein (AR1) with the silencing sequence. The specification further demonstrates that the silencing sequence may be in the sense orientation, may be in the antisense orientation, and/or may be frame-shifted. The application further states that the silencing sequence may be as short as 150 nucleotides, and specific working examples are provided with sequences that are 403, 623, 786 and 1492 bp in length (Specification, *e.g.*, at page 6, lines 3- 6). As discussed above, those skilled in the art will appreciate that the

degree of sequence similarity will generally vary inversely with the length of the silencing sequence carried by the vector. The method may be carried out with any plant, and an illustrative listing of plants is provided at page 16 (line 22) to page 17 (line 20) of the Specification. Specific working examples are provided with various TGMV constructs, in sense and anti-sense orientation, in *N. benthamiana*.

Accordingly, Applicant reiterates that the specification provides extensive written description, and the outstanding rejection appears to be questioning whether the invention can be made and used as described. It is unclear to Applicant what further written description in the specification is deemed necessary. If the outstanding rejection is maintained, it is respectfully requested that the Examiner clarify this rejection so that the Applicant may better address the Examiner's concerns and to provide a more complete record in the event of an appeal.

As a final point, the Applicant notes that the cited Kridl et al. patent only provides working examples involving construction of an African Cassava Mosaic Virus (ACMV) expression cassette, and does not demonstrate expression of any transgene sequence therefrom. Claims 1 and 2 of Kridl et al. are generic as to the geminivirus and the transgene of interest to be expressed. As the Kridl et al. reference is an issued U.S. patent, the USPTO clearly deemed that the specification of Kridl et al. provided adequate written description under § 112, paragraph 1, for generic claims. As the present application provides greater written description than the Kridl et al. patent and actual working examples of gene silencing using multiple transgene constructs, it would be contradictory to maintain that the present application does not satisfy the written description requirement found in §112, paragraph 1 in view of the issuance of Kridl et al. **and** further in view of the application of Kridl et al. under § 102 in the outstanding Office Action.

For the reasons set forth in detail above, the Applicant submits that the claims are supported by specification as filed and respectfully request that the outstanding rejection for lack of written support be withdrawn.

III. Enablement.

Claims 1-6, 8-9, 11-16, 18-19, 21, 31-32 and 35-60 stand rejected under 35 U.S.C. § 112, first paragraph as non-enabled. Specifically, the Office Action states that the specification is "enabling for the silencing of the magnesium chelatase gene and the luciferase gene in *N. benthamiana* using a TGMV vector", but argues that it does not "reasonably provide enablement for any and all geminivirus silencing vectors comprising any and all geminivirus genomes." (Office Action, page 4, para. 3). Claim 61 appears to be free of this rejection, but is not explicitly acknowledged as such. If this rejection is maintained it is respectfully requested that the Examiner clarify the status of Claim 61.

As an initial point, the Office Action states that the claims are drawn to geminivirus vectors comprising sequences having at least 60% sequence similarity to a plant gene. The Applicant respectfully notes that some claims (*i.e.*, Claims 42-49 and 62) specifically recite TGMV or ACMV. Other claims specifically recited at least 80%, 90% or 95% sequence similarity (*i.e.*, Claims 42-43, 46-47, and 59-62). Further, the independent claims have been amended herein to recite at least 80% sequence similarity. If the present rejection is maintained, it is respectfully requested that the Examiner specifically indicate why claims containing these recitations (*i.e.*, TGMV, ACMV and at least 80%, 90% or 95% sequence similarity) are not enabled.

The working examples in the present application demonstrate silencing of the magnesium chelatase gene and a luciferase transgene in *N. benthamiana* with a variety of different sense and antisense fragments in a TGMV derived construct. The Peele et al. and Turnage et al. manuscripts demonstrate that the constructs and methods of the invention may be employed to silence a variety of other unrelated genes (green fluorescent protein, PCNA, phytoene desaturase genes, magnesium chelatase) in other plants (*Arabidopsis*), and using silencing vectors derived from other

geminiviruses that are only distantly related to TGMV (Cabbage Leave Curl Virus).

The present application demonstrates that gene fragments in the range of approximately 400 to 1500 bp (in sense or antisense direction) can effect gene silencing from a geminivirus silencing vector. The Peele et al. manuscript also supports the breadth of the present claims in that they confirm that shorter sequences may also be used to produce gene silencing (51, 92, 154 and 180 bp).

Moreover, the cited Atkinson et al. reference demonstrates silencing of a further gene (a chalcone synthase A gene) using a very distantly related geminivirus (tobacco yellow dwarf virus; TYDV), which is a monopartite geminivirus) in yet another plant species (petunia).

It is unclear to the Applicant how there is any objective basis for maintaining the outstanding enablement rejection in view of the evidence of gene silencing by the vectors and methods of the invention in a number of distantly related species (*Arabidopsis*, *Nicotiana benthamiana*, and petunia) using several distantly related geminiviruses (TGMV, CbLCV and TYDV) with various unrelated target genes (magnesium chelatase, luciferase, green fluorescent protein, chalcone synthase A gene, PCNA, and phytoene desaturase genes). Moreover, silencing fragments ranging from approximately 50 to 1500 bp have been demonstrated to produce gene silencing. Finally, silencing was observed with sequences having less than complete sequence similarity to endogenous plant sequences.

Thus, the Applicant has demonstrated that the claimed invention works as described in the specification across a variety of target genes, geminiviruses, plant species, and lengths of silencing sequences. The Applicant asserts that the evidence that has been presented is sufficient to support the breadth of the present claims. It is not possible, nor is it required under the legal standards of enablement, that the Applicant demonstrate silencing in all possible species, with all possible geminiviruses using all possible transgenes. All that is required is that the proffered evidence be

representative of the claimed genus. By any objective standard, the Applicant has clearly satisfied the enablement requirement.

With respect to the arguments in the Office Action regarding Covey et al. and Neuhaber et al., the Applicant maintains that patentability does not rely on elucidation of the molecular basis of gene silencing. It is sufficient that the invention may be made and used in accordance with the description in the application. The Applicant has presented ample evidence that the skilled worker may make and use the invention to effect gene silencing as described in the application. There will undoubtedly be continuing work to understand the interaction between plant viruses and their hosts (*e.g.*, viral silencing and plant anti-silencing mechanisms); however, these studies are irrelevant to the question of patentability. It is sufficient that the Applicant has presented clear evidence that the claimed invention works as described in the application; which standard has been satisfied in the instant application.

Moreover, the Office Action appears concerned that the degree and pattern of gene silencing is not uniform (citing Voinnet), *e.g.*, there may be variations in the tissues/cells that exhibit silencing and the level of silencing. Again, these concerns are inapposite to the enablement inquiry. Enablement does not require that maximal or uniform levels of silencing be observed across all cells and tissues in all plant species with all geminivirus constructs, and the claims do not recite any such features.

The present invention does not require uniform gene silencing or gene silencing in all plant tissues. Further, the Applicant asserts that, using the present specification as a guide, it would be routine for the ordinarily skilled worker (*e.g.*, a laboratory technician) to screen silencing fragments of different lengths in a variety of plant cells to identify those that produce the desired levels of gene silencing in the desired target cells. The cited references merely articulate some factors or parameters that the skilled worker would have to consider in practicing the claimed invention. As these factors are well-known in the art, it would not be an undue burden to vary those

parameters so as to identify geminivirus constructs and methods having the desired properties.

In addition, the constructs and methods described by Voinnet et al. are quite different from the presently-claimed silencing constructs and methods. Voinnet et al. expressed, in isolation, the AL2 (called the AC2) protein of ACMV from an *Agrobacterium* vector. This report utilizes a very complex system involving a transient assay using *Agrobacterium* infiltration of AL2 carried by a potato virus X vector. As an initial matter, *Agrobacterium* itself has effects on gene expression. In addition, Voinnet et al. express the AL2 protein in isolation, *i.e.*, without the other geminivirus proteins. It is known in the art that expression of AL2 alone has different effects than expression within the context of the virus, presumably because other viral proteins interact with AL2 to moderate its effects. For example, Hartitz et al., (1999) *Virology* **263**:1-14 (copy enclosed), has observed that expression of AL2 is toxic to plants (*see*, page 8, second paragraph, "However, cells expressing full-length TrAP [AL2 gene product] grew much more slowly and produced much smaller colonies than control cells or cells expressing other TrAP fusion proteins." Likewise, Sunter et al., (2001) *Virology* **285**:59-70 (copy enclosed), have stated that "[a] truncated AL2 gene was chosen to study the effects of TrAP [AL2 gene product] in the absence of its transcriptional activation domain, which has been mapped to the C-terminal amino acids 115-129, and because of previously observed toxicity of the full-length wild-type AL2 gene (Hartitz et al., 1999; unpublished results)." (Sunter et al., page 60, left column, first paragraph of "Results" section).

Accordingly, the results of Voinnet et al., which are based on expression of AL2 alone in an artificial system, are not germane to the presently-claimed constructs and methods. It is known in the art that expression of AL2 alone is toxic, and the results observed by Voinnet would not be applicable to the present invention, which does not express AL2 in isolation of other geminivirus proteins.

Finally, the Examiner's concerns with respect to Voinnet, Covey and Neuhaber have been addressed by the abundant evidence provide by the Applicant above that gene silencing of a large number of unrelated genes may be achieved with a number of distantly related geminiviruses, and in widely divergent plant species.

In view of the foregoing, the Applicant submits that the claims satisfy the enablement requirement of § 112, first paragraph, and respectfully request that the rejection on this basis be withdrawn.

IV. Claims 42-49, 62-63 and 65.

Claims 42-49, 62-63 and 65 satisfy the written description and enablement requirements of § 112, first paragraph for the reasons set forth in detail in the preceding two sections. In addition, the Applicant notes that these claims specifically recite ACMV or TGMV. Claim 65 further specifically recite "a cell from a species of *Nicotiana*." Accordingly, the Applicant submits that these claims that are directed to specific embodiments cannot reasonably be construed as overbroad, and are clearly supported and enabled by the specification. In particular, with respect to Claims 42-43, 46-47, 62-63 and 65, the working examples demonstrate gene silencing with TGMV in *Nicotiana*.

In view of the foregoing, the Applicant requests that the rejections of Claims 42-49, 62-63 and 65 for lack of written support and non-enablement be withdrawn.

V. The Claims are Patentable over Kridl et al.

Claims 1-9, 11-16, 18-19, 21, 31-32, 35-41, 44-45, 48-49 and 50-61 stand rejected under 35 U.S.C. § 102(b) over U.S. Patent No. 5,589,379 (Kridl et al.). Applicant notes that Claims 42, 43, 46 and 47 are free of this rejection. The outstanding rejection will be addressed below with respect to pending Claims 1-6, 8, 9, 11-16, 18, 19, 21, 31, 32 and 36-58 and 60-65.

The Kridl et al. patent describes an African Cassava Mosaic Virus expression vector for expressing a polypeptide in target cells. This reference

does not disclose or suggest a silencing vector as recited by Claims 1-6, 8, 9, 11-16, 18, 19, 21, 31, 32 and 36-58 and 60-65. The silencing vectors of the present invention may contain a coding or non-coding region of a gene, in the sense or anti-sense orientation; the silencing sequence may even be frame-shifted (*see, e.g.*, Specification at Examples 1-3). Kridl et al. does not disclose a silencing vector having these properties.

Moreover, all of the independent claims have been amended to recite that the heterologous DNA has at least 80% sequence similarity to an endogenous plant gene. Claims 60-61 recite at least 90% or 95% sequence similarity, respectively. Kridl et al. does not disclose a silencing vector comprising a heterologous DNA having the recited levels of sequence homology to an endogenous plant gene.

A. Claims 1-11.

Claims 1-11 are novel and unobvious over Kridl et al for the reasons set forth above. In addition, Claim 1 has been amended to recite that the silencing vector comprising "the geminivirus AL1, AL2 and AL3 coding sequences", and "said heterologous DNA is constitutively expressed and said AL1, AL2 an AL3 coding sequences are bidirectionally transcribed from said geminivirus silencing vector." New Claim 62 contains these same recitations. None of these recitations is disclosed or suggested by the cited Kridl reference.

Kridl et al. describes a system that attempts to achieve regulated expression of AL2 and, consequently, a transgene driven by the coat protein promoter. AL1 and AL2 are early gene products that mediate viral replication, AL2 is a transactivating protein that turns on expression of the coat protein (AR1) and geminivirus movement proteins (*i.e.*, late protein expression). Thus, AL2 is the switch between early and late protein expression. AL1, AL2 and AL3 are expressed by the geminivirus as a polycistronic message, with overlap between the three reading frames, driven by the AL1 promoter. Kridl et al. describes putting AL2 under the control of an inducible/regulatable

promoter so as to regulate expression of AL2 and, therefore, the coat protein (AR1) and movement proteins. Kridl further describes a transacting expression cassette, which comprises the AL2 coding sequences operably associated with an inducible/regulatable promoter, and a transactivatable expression cassette, which expresses the transgene of interest from the coat protein (AR1) promoter.

Thus, Kridl et al. describes a system for achieving regulated expression of AL2 so as to regulate the AR1 (coat protein) promoter and the transgene of interest. In contrast, Claim 1 (and dependents) and Claim 62 recite constitutive expression of the foreign sequence.

Moreover, Claim 1 (and dependents) and Claim 62 recite that the geminivirus silencing vector comprises the AL1, AL2 and AL3 coding sequence, which are "bidirectionally transcribed" from the silencing vector. Kridl et al. does not describe a construct having these properties. At Col 5, line 8 to Col. 6, line 34, Kridl et al. describes the transacting and transactivatable constructs. In one embodiment, the transacting construct comprises an AL2 coding sequence operably associated with a regulatable promoter and the transactivating construct comprises a transgene operably associated with the coat protein promoter. In a second embodiment, the transactivatable construct is a modified geminivirus vector in which the AL2 gene has been inactivated. In a third embodiment, a modified geminivirus construct is made in which a regulatable promoter is inserted 3' to the AL1 region and 5' to the AL2 coding region, presumably to achieve regulated expression of AL2. The Applicant submits that this latter embodiment is inoperable as the AL1, AL2 and AL3 coding regions are overlapping and are transcribed as a polycistronic message (*see, e.g.*, Figure 1A of the present application, and accordingly there is no region that is 3' to AL1 and 5' to AL2. Moreover, even if this type of construct were feasible, the disassociation of expression of AL2 and AL3 would interfere with normal replication of the construct.

Accordingly, Kridl et al. does not describe any geminivirus construct comprising the AL1, AL2 and AL3 coding sequences, wherein the coding sequences are bidirectionally transcribed from the geminivirus vector.

Moreover, new Claim 62 recites "TGMV AL1, AL2 and AL3 coding sequences operably associated with an AL1 promoter." As described in some detail above, Kridl does not describe driving expression of AL2 from the AL1 promoter; indeed, the whole focus of Kridl et al. is to remove AL2 expression from the control of the AL1 promoter.

Accordingly, for the reasons set forth in detail above, Applicants submit that the subject matter of Claims 1-11 and 62 is neither disclosed nor suggested by Kridl et al., and respectfully request that the outstanding anticipation rejection be withdrawn.

B. Claims 36-37.

The Applicant submits that Claims 36-37 are novel and unobvious over Kridl et al. for the reasons set forth above. In addition, these claims recite a geminivirus silencing vector where the silencing sequence has at least 80% sequence similarity to an endogenous plant gene and where the silencing fragment is in the sense orientation and comprises only a fragment of the full-length plant gene. Kridl et al. describes expression vectors. However, Kridl et al. provides absolutely no motivation or suggestion to one of ordinary skill in the art to generate the recited geminivirus vector expressing only a portion of the sense sequences of an endogenous plant gene. Based on the teachings of Kridl et al. the ordinary skilled worker would only be motivated to express a full-length sequence in the sense orientation, *e.g.*, to express a polypeptide (Kridl et al., Col. 11, lines 13-15). There would be no suggestion to express less than the full-length sense sequence as Kridl et al. provides no suggestion that expressing only a fragment of the sense sequence would have any useful effect. In contrast, the present inventor has unexpectedly found that gene silencing can be achieved with a geminivirus silencing vector comprising a

sequence that has substantial sequence homology to a fragment of the sense orientation sequences of an endogenous plant gene.

Accordingly, the Applicant submits that the subject matter of Claims 36-37 is clearly not anticipated or rendered obvious by Kridl et al. as this reference does not disclose or suggest a geminivirus construct comprising a fragment of an endogenous plant gene in the sense orientation. One of ordinary skill in the art would only be motivated by the Kridl et al. reference to express a full-length sense construct for the purposes of gene expression.

C. Claims 38-41.

The Applicant further submits that Claims 38-41 are patentable over Kridl et al. These claims as amended recite a silencing vector comprising "heterologous DNA having at least 80% sequence similarity to a coding region of a gene endogenous to a plant", wherein the silencing sequence is further in the anti-sense orientation.

Kridl et al. focuses on the expression of full-length sense constructs for the purpose of gene expression, not silencing. Kridl et al. does make a speculative statement (at Col. 11, lines 13-20) that antisense constructs to "genomic sequences" may be used to inhibit transcription. The genomic sequence "may be an open reading frame, an intron, a noncoding leader sequence, or any other sequence where the complementary sequence inhibits transcription, messenger RNA processing, for example, splicing or translation." In other words, Kridl et al. does not teach or suggest use of an antisense sequence that is complementary to the coding region of a plant gene to inhibit transcription thereof, as recited by Claim 38-41.

Accordingly, for this additional reason, the Applicant submits that the silencing constructs of Claims 38-41 are novel and unobvious over Kridl et al.

D. M thod Claims 50-61.

The Applicant submits that method Claims 50-61 are patentable over Kridl et al. for the reasons presented above for the silencing vector claims.

The methods of Claims 50-61 may further be distinguished from this reference in that these methods are drawn to methods of gene silencing, whereas Kridl et al. is primarily concerned with methods of expressing a heterologous nucleotide sequence of interest using a geminivirus transfer vector. Kridl et al. merely speculates that antisense sequences to non-coding regions may be used to inhibit transcription (Kridl et al., Col. 11, lines 13-20). It was not previously appreciated in the art, prior to the Applicant's discovery, that a virus that is episomal, has a DNA genome, and locates to the nucleus of the host cell could be used for gene silencing as had been previously observed with cytoplasmic RNA viruses. Thus, one of ordinary skill in the art would not have been motivated by Kridl et al. to use a geminivirus based vector for the purpose of gene silencing. Further, one of ordinary skill in the art would not have appreciated from Kridl et al. that a geminivirus construct comprising a heterologous sequence having substantial sequence similarity to the coding region of a plant gene, in either the sense or anti-sense direction, could be employed for gene silencing.

Accordingly, for this additional reason, the Applicant submits that the methods of Claims 50-61 are novel and unobvious over Kridl et al.

E. Claims 54-57.

Claims 54-57 may be further distinguished from Kridl et al. in that these claims specifically recite methods of achieving "systemic silencing" in plants. As described in the present application at page 10 (lines 18-25) and in Example 3, the Applicant determined that the inventive geminivirus silencing vectors may be used to achieve systemic silencing in cells that are remote from the site of initial inoculation of the DNA silencing construct. Kridl et al. does not disclose or suggest that a method of achieving systemic gene silencing in plants using a geminivirus silencing vector.

Accordingly, for this further reason, the Applicant submits that the methods of Claims 54-47 are both novel and unobvious over Kridl et al.

F. **Claims 63 and 65.**

Applicants submit that Claims 63 and 65 are patentable for all of the reasons discussed above. In addition, these claims are distinguishable from Kridl et al. on the following basis.

New Claim 63 depends from Claim 42, which is free of the outstanding rejection over Kridl et al. Accordingly, the Applicant submits that Claim 63 is also free of this rejection.

New Claim 65 does not depend from Claim 42, but like Claim 42 recites "TGMV". Accordingly, the Applicant submits that new Claim 65 should be free of the rejection over Kridl et al. as well.

G. **Claims 64 and 65.**

New Claims 64 and 65 are patentable over the cited reference for the reasons discussed above. In addition, these claims may be further distinguished from Kridl et al. in that they recite a method of gene silencing comprising providing a nucleic acid sequence(s) encoding the geminivirus movement proteins to the cell. Kridl et al. does not in any way disclose or suggest providing the movement proteins or the geminivirus B component to the cell/plant and, accordingly, does not anticipate or render obvious the subject matter of Claims 64 and 65. As described in the present specification, the degree of gene silencing is more extensive in the presence of the geminivirus movement proteins, and this advantageous effect is neither disclosed nor described by Kridl et al.

Accordingly, for the reasons set forth above, the Applicant submits that the subject matter of Claims 64 and 65 is both novel and unobvious over Kridl et al., and respectfully request that the rejection over this reference be withdrawn.

VI. Conclusion.

The points and concerns raised by the Examiner in the outstanding Office Action having been addressed in full, it is respectfully submitted that this application is in condition for allowance, which action is respectfully submitted. Should the Examiner have any remaining concerns, it is requested that the Examiner contact the undersigned representative to expedite the prosecution of this application.

Respectfully submitted,

Karen A. Magri
Registration No. 41,965

Enclosures: Hartitz et al.
Sunter et al.
Clean version of pending claim set

Customer Number:

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CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner for Patents, Washington, DC 20231, on April 10, 2002.

Sloan Hobbs

V rsion with Markings to Show Chang s Mad

1. (Twice Amended) A geminivirus silencing vector comprising a geminivirus genome comprising [which contains]:

the geminivirus AL1, AL2 and AL3 coding sequences,

heterologous DNA, said heterologous DNA having at least 80% [60%] sequence similarity to a gene endogenous to a plant that occurs naturally in the plant genome,

wherein said heterologous DNA is constitutively expressed and said AL1, AL2 and AL3 coding sequences are bidirectionally transcribed from said geminivirus silencing vector, and

wherein said geminivirus silencing vector silences expression of the endogenous plant gene upon introduction into a plant cell.

8. (Amended) A vector according to claim 1, wherein said heterologous DNA has at least 80% [60%] sequence similarity to a fragment of said endogenous plant gene.

9. (Twice Amended) A vector according to claim 1, wherein said heterologous DNA has at least 80% [60%] sequence similarity to the entire coding region of endogenous plant gene.

12. (Twice Amended) A DNA construct comprising a geminivirus genome, wherein the DNA encoding the geminivirus coat protein has been replaced in part or in total with heterologous DNA having at least 80% [60%] sequence similarity to an endogenous plant gene that occurs naturally in the plant genome.

18. (Twice Amended) A DNA construct according to claim 12, wherein said heterologous DNA has at least 80% [60%] sequence similarity to a fragment of said endogenous plant gene.

19. (Twice Amended) A DNA construct according to claim 12, wherein said heterologous DNA has at least 80% [60%] sequence similarity to the entire coding region of said endogenous plant gene.

36. (Amended) A geminivirus silencing vector comprising a geminivirus genome which contains heterologous DNA, said heterologous DNA having at least 80% [60%] sequence similarity to a fragment of a gene endogenous to a plant, wherein the heterologous DNA sequence is inserted into the silencing vector in the sense orientation, and wherein said geminivirus silencing vector silences expression of the endogenous plant gene upon introduction into a plant cell.

38. (Amended) A geminivirus silencing vector comprising a geminivirus genome which contains a heterologous DNA, said heterologous DNA having at least 80% [60%] sequence similarity to a coding region of a gene endogenous to a plant, wherein the heterologous DNA sequence is inserted into the silencing vector in the antisense orientation, and wherein said geminivirus silencing vector silences expression of the endogenous plant gene upon introduction into a plant cell.

40. (Amended) A DNA construct comprising a geminivirus genome, wherein the DNA encoding the geminivirus coat protein has been replaced in part or in total with heterologous DNA having at least 80% [60%] sequence similarity to a coding region of a gene endogenous to a plant, and wherein the heterologous DNA sequence is inserted into the geminivirus genome [silencing vector] in the antisense orientation.

42. (Amended) A geminivirus silencing vector comprising a Tomato Golden Mosaic Virus (TGMV) genome which contains heterologous DNA, said heterologous DNA having at least 80% [60%] sequence similarity

to a gene endogenous to a plant, wherein said geminivirus silencing vector silences expression of the endogenous plant gene upon introduction into a plant cell.

44. (Amended) A geminivirus silencing vector comprising an African Cassava Mosaic Virus (ACMV) genome which contains heterologous DNA, said heterologous DNA having at least 80% [60%] sequence similarity to a gene endogenous to a plant, and wherein said geminivirus silencing vector silences expression of the endogenous plant gene upon introduction into a plant cell.

46. (Amended) A DNA construct comprising a Tomato Golden Mosaic Virus (TGMV) genome, wherein the DNA encoding the TGMV coat protein has been replaced in part or in total with heterologous DNA having at least 80% [60%] sequence similarity to an endogenous plant gene.

48. (Amended) A DNA construct comprising an African Cassava Mosaic Virus (ACMV) genome, wherein the DNA encoding the ACMV coat protein has been replaced in part or in total with heterologous DNA having at least 80% [60%] sequence similarity to an endogenous plant gene.

50. (Amended) A method of silencing the expression of an endogenous plant gene in a plant cell, comprising inoculating said plant cell with a geminivirus silencing vector comprising a geminivirus genome which contains heterologous DNA, said heterologous DNA having at least 80% [60%] sequence similarity to a gene endogenous to a plant.

52. (Amended) A method of silencing the expression of an endogenous plant gene in a plant cell, comprising inoculating said plant cell with a DNA construct comprising a geminivirus genome, wherein the DNA encoding the geminivirus coat protein has been replaced in part or in total with

heterologous DNA having at least 80% [60%] sequence similarity to an endogenous plant gene.

54. (Amended) A method of systemically silencing expression of an endogenous plant gene in a plant, comprising inoculating said plant [cell] with a geminivirus silencing vector comprising a geminivirus genome which contains heterologous DNA, said heterologous DNA having at least 80% [60%] sequence similarity to a gene endogenous to a plant.

56. (Amended) A method of systemically silencing expression of an endogenous plant gene in a plant, comprising inoculating said plant [cell] with a DNA construct comprising a geminivirus genome, wherein the DNA encoding the geminivirus coat protein has been replaced in part or in total with heterologous DNA having at least 80% [60%] sequence similarity to an endogenous plant gene

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